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Metallothionein and manganese concentrations in breast cancer and mastopathic tissues

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Abstract

Metallothionein (MT) takes part in the homeostasis of the metals which are necessary for proper metabolism, and they also take part in the detoxication of toxic metals from the tissues. Additionally, they protect the tissues from the effects of reactive, oxygen species, radiation, mutagens, and from electrophilic pharmacologic agents used in cancer therapy. Manganese (Mn) – an essential microelement - seems to be very important during development. Mn takes a part as cofactor of the enzymes regulating metabolism. This microelement also participates in reduction-oxidation reactions. An example of such a reaction is the superoxide dismutase (MnSOD) activity, with manganese as the enzyme component. It is thought that manganese and manganese superoxide dismutase play an important role in the differentiating cells. The aim of this work was to evaluate whether the neoplastic process changes the values of metallothionein and manganese. The examined material consisted of samples of breast tumour taken during surgery. The level of the metallothionein was determined by the cadmium- hemoglobin affinity assay, using the cadmium isotope (^{109}Cd); the concentration of manganese was determined by atomic absorption spectrometry. It was found that metallothionein and manganese concentration in breast cancer was higher in comparison with mastopathic lesions of the breast. Neoplastic cells contained more metallothioneins (by 330%) and more manganese (by 25%). Negative correlations were found between the amount of metallothionein and the level of manganese in mastopathic lesions ($r = -0.57$, $p < 0.05$), and in breast cancer ($r = -0.32$, $p > 0.05$). The relatively high level of metallothionein and manganese in the neoplastic tissues indicates the important role may play in the metabolism of cancer cells. Negative correlation between the concentration of metallothionein and manganese ions found in mastopathic tissues and breast cancer indicates that their roles are opposite.

Key words

metallothionein, manganese, breast cancer, mastopathic tissues

INTRODUCTION

Metallothionein (MT) is a widespread protein in the animal world, characterised by a great invariability of their structure. While isolated from various animals, they only slightly differ from one another in amino acid composition. The number of amino acids is constant in every animal group – 60 (or 61) amino acids, 20 of which are the cysteins residues, which comprises over 30% of the composition of the amino acids. Such a high amount of cysteins determines the functions of metallothionein. Metallothioneins take part in the homeostasis of the ions of the metals necessary for the proper metabolism of an organism; they also take part in the detoxication of the tissues from toxic metals, and protect them from reactive oxygen species, radiation, electrophilic pharmacological agents used in the cancer therapy, as well as protecting against mutagens [1-4].

Manganese (Mn) seems to be a very important microelement during development. This microelement participates in the reduction-oxidation reactions due to its chemical properties (several oxidation stages). An example of such a reaction is the superoxide dismutase (MnSOD) activity with manganese as an enzyme component. It is thought that manganese and manganese superoxide dismutase play an important role in the differentiating cells [5, 6].

In the oncogenic process, owing to external factors, some changes take place in the organism. Some of them include a change in the pace of metabolism and of microelement distribution [7].

An increase in metallothionein concentration in cancerous cells at an early stage of tumour development, as stated by many authors, suggests that the evaluation of metallothioneins may serve as an early biomolecular marker of a neoplastic process [8, 9].

These observations have served as a basis for research, the objectives of which were to evaluate whether the neoplastic process changes the values of metallothionein and manganese in breast cancer.

MATERIAL AND METHODS

The examined material consisted of samples of breast tumour removed during surgery. Only those cases qualified which did not undergo chemo- or radiotherapy prior to the operation. Also excluded from further study were patients undergoing microelement supplementation.

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Breast cancer constituted of invasive ductal tumorus (*carcinoma ductale invasivum*), classified as $T_1N_0M_0$ and $T_2N_1M_0$ (n = 20, age: 42-65) [10]. The control group consisted of benign breast lesions – fibro-cystomatic mastopathies (*mastopathia fibrosa cystica*, n = 20, age 49-60) [10]. All experimental material was obtained from the Oncological Clinic of the Medical University in Lublin.

Metallothionein level. The concentration of metallothionein was determined by the cadmium-haemoglobin affinity assay, using the cadmium ^{109}Cd isotope (Du Pont, USA). The tissues were homogenised in 4 volumes of 10 mM Tris-HCl pH 7.4, then the homogenate was centrifuged at 10,000 g for 10 min. and the supernatant heated for 2 min. at 100°C in a water bath. Samples were centrifuged at 10,000 g for 2 min. in order to remove precipitated proteins. Carrier-free ^{109}Cd was dissolved in 10 mM Tris-HCl buffer pH 7.4 adding $CdCl_2$ to yield a Cd concentration of 2.0 μg/ml and radioactivity of 1.0μCi/ml. Next, 200μl of this ^{109}Cd solution was mixed with 200μl of homogenate and incubated for 10 min. Then, 100 μl of a 2% bovine haemoglobin solution (Sigma, USA) was added to the tube, heated at 100°C in the water bath for 2 min, and 100 μl of the 2% haemoglobin solution was again added. After 2 min. heating, centrifugation was performed under the same conditions. The radioactivity of the supernatant samples (500μl) were measured using gamma-counter (Beckman LS6000TA, USA). Blank samples (buffer instead of tissue sample) and total activity samples (buffer instead of haemoglobin) were run simultaneously [11].

Table 1. Level of metallothionein (in μg/g of wet tissue) and manganese (in mg/kg of wet tissue) in mastopathic cells and breast cancer cells.

Studied parameters	Mastopathic tissues n = 20 (mean value and standard deviation)	Breast cancer n = 20 (mean value and standard deviation)	Significance level
Metallothionein	6.29 ± 1.87	21.46 ± 6.38	$p < 0.05$
Mn	0.23 ± 0.09	0.31 ± 0.12	$p < 0.05$

Level of manganese. The weighed samples of each tissue were washed out in the physiological solution and then subjected to dessication for 72 h at 80°C, ashed at 450°C, and then dissolved in concentrated HCl, which had been mixed 1 : 1 with H_2O (v/v). Determination of manganese was performed using Atomic Absorption Spectrometry (AAS; Pye Unicam SP 192) [12].

Statistical Analysis. Statistical analysis of the results was conducted using an SPSS 8,0 pack. To evaluate the significance of variable discrepancies of standard distribution, the Mann-Whitney U-test for independent trials was used. The interdependencies of particular parameters in the groups were examined by means of Pearson correlation coefficient, and linear regression. The assumed hypotheses were verified on the significance level of $p < 0.05$.

RESULTS

Table 1 shows the metallothionein concentration in μg/g of wet tissue; manganese (Mn) concentration is shown in mg/kg of wet tissue. The breast cancer group, when compared with the mastopathic lesions group, contained more metallothioneins (about 300%) and more manganese (about 25%). Significant differences were found in the content of metallothioneins ($p < 0.05$) and in the content of manganese ($p < 0.05$).

The correlation of metallothionein concentration and manganese content in the examined groups was negative: -0.57, $p < 0.05$ for mastopathic cells, and -0.32, $p > 0.05$ for breast cancer (Fig. 1, Fig. 2).

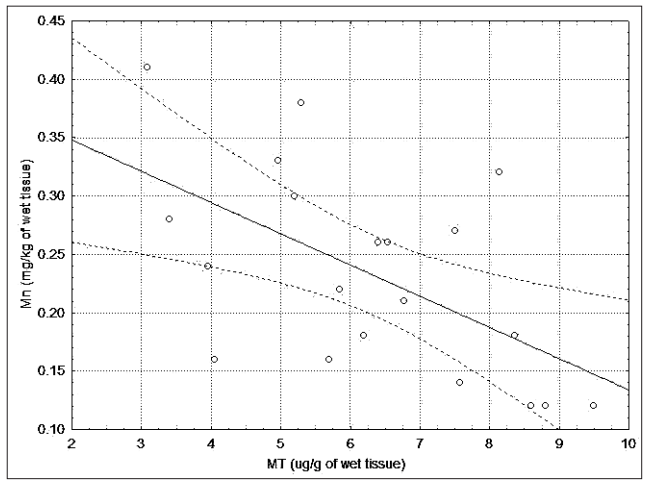

Figure 1. The correlation coefficient between the levels of the studied parameters in mastopathic tissues

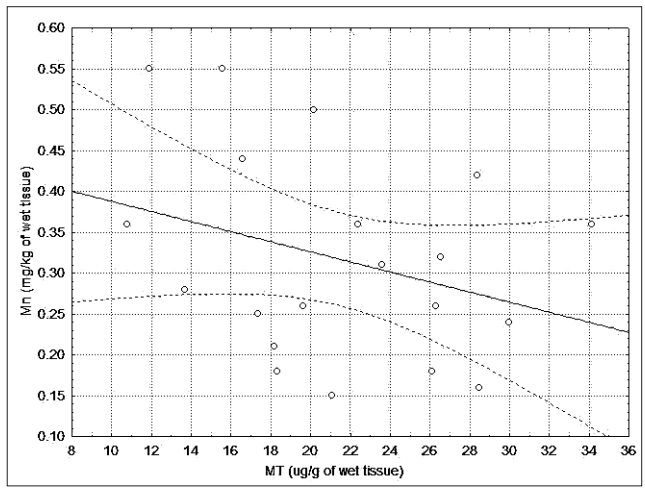

Figure 2. The correlation coefficient between the levels of the studied parameters in breast cancer

DISCUSSION

Living organisms have developed mechanisms of utilising vital trace elements, such as zinc and copper, and reducing to the minimum the toxic influence of heavy metals like cadmium, mercury and lead. Metallothionein, intracellular proteins rich in cysteine residues, have the ability to bind an excess of metal ions, thus regulating the number of their free ions [13]. The binding of metals takes place via cysteine residues.

In mammals, the highest concentration of MT can be found in the liver and kidneys (10-15 μg of MT per gram of wet tissue). This indicates the important role of these organs in the detoxication processes and in the homeostasis of metal ions. According to the literature data, in the other tissues the concentration of MT is significantly lower (1-2 μg of MT per gram of wet tissue) [14, 15].

The concentration of metallotionein in breast cancer is relatively high, and in many cases the concentration is significantly higher in the liver and kidneys. The synthesis and intracellular distribution of MT is a very important aspect in oncology, because these proteins not only indicate a protective role in environmental factors, but also are responsible for the cell's resistance to pharmacological medications [3, 16, 17].

The intensive expression of intracellular MT and manganese has been shown in many kinds of human and animal neoplasms [18-23]. The studies have shown that the following factors induce the synthesis of MT in the neoplasm cell: tumour necrosis factor (TNF), interferon α, interleukin-1, and interleukin-2. The same factors induce an increased concentration of manganese in cancer cells.

Manganese (Mn) seems to be very important microelement during development. Mn takes a part as a co-factor of the enzymes regulating the metabolism of glucose and other carbohydrates, as well as lipids and proteins synthesis. Mn is also needed for the synthesis of proteoglycans and glycoprotein. It is a crucial component for bone and nerves system formation [24]. This microelement also participates in the reduction-oxidation reactions due to its chemical properties (several oxidation stages). An example of such a reaction is the superoxide dismutase (MnSOD) activity, with manganese as enzyme component. It is thought that manganese and manganese superoxide dismutase play an important role in the differentiating cells [5, 25, 26].

The correlation coefficient of the studied parameters proved an interrelation between the levels of manganese and the content of metallothionein. The negative correlation between the concentration of metallothionein and manganese ions found in mastopathic tissues and breast cancer indicates that their roles may be reciprocal. In normal conditions, metallothionein isolated from the liver contains mainly zinc and copper [15,27].

Although the factors inducing the synthesis of MT are known, the mechanisms responsible for MT distribution have not yet been well documented. The synthesis of the MT is induced by the ions of metals, hormones, inflamatory factors, free radicals, physical stress and some pharmacological agents [28]. The presence of the regulatory sequence in the gene for MT (metal responsive element – MRE and glycocortycoid responsive element – GRE), enables direct induction of the transcription with the participation of the metals' ions and glycocortycoid. The other factors stimulate the synthesis of MT in an indirect way [9,27,29]. In the case of all the factors, there are differences depending on the kind of tissue and the kind of induced metallothionein.

CONCLUSION

- The high level of metallothionein and manganese in the neoplastic tissues indicates the important role they play in the metabolism of cancer cells.
- The negative correlation between the concentration of metallothionein and manganese ions found in mastopathic tissues and breast cancer indicates that their roles are reciprocal.

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